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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Anne N. Murphy *et al.*
Application No.	:	09/709,785
Filed	:	November 3, 2000
For	:	COMPOSITIONS AND METHODS FOR DETERMINING INTERACTIONS OF MITOCHONDRIAL COMPONENTS, AND FOR IDENTIFYING AGENTS THAT ALTER SUCH INTERACTIONS

Action	:	Arun K. Chakrabarti
Art Unit	:	1655
Docket No.	:	660088.433C1
Date	:	July 30, 2002

Commissioner for Patents
Washington, DC 20231

AMENDMENT AND RESPONSE

Commissioner for Patents:

In response to the Office Action mailed on January 30, 2002, please extend the period for response three months to expire on July 30, 2002. A petition for extension of time and the requisite fee are enclosed.

Please amend the application as follows:

In the specification:

Please amend the paragraph that starts at page 28, line 10 to read as follows:

Sub C4

As described herein, the invention provides ANT and CypD fusion proteins comprising ANT or CypD polypeptides fused to an additional functional or non-functional polypeptide sequence that permits, for example by way of illustration and not

limitation, detection, isolation and/or purification of the ANT and CypD fusion proteins. For instance, an additional functional polypeptide sequence may be an energy transfer molecule polypeptide as provided herein. ANT and CypD fusion proteins described herein may be detected by FRET, fluorescence, phosphorescence, bioluminescence, or chemiluminescence, and include fusion proteins that may in certain embodiments be detected, isolated and/or purified by protein-protein affinity (*e.g.*, receptor-ligand), metal affinity or charge affinity methods. In certain other embodiments the subject invention fusion proteins may be detected by specific protease cleavage of a fusion protein having a sequence that comprises a protease recognition sequence, such that the ANT and CypD polypeptides may be separable from the additional polypeptide sequence. In particularly preferred embodiments, for example, each ANT and/or CypD polypeptide sequence is fused in-frame to an energy transfer molecule polypeptide sequence. Other polypeptide sequences present in ANT and CypD fusion proteins may facilitate affinity detection and isolation of ANT and CypD polypeptides and may include, for example, poly-His or the defined antigenic peptide epitopes described in U.S. Patent No. 5,011,912 and in Hopp et al., (1988 *Bio/Technology 6*:1204) (*e.g.*, FLAG® epitope tag DYKDDDDK, SEQ ID NO:__), or the XPRESS™ epitope tag (DLYDDDDK, SEQ ID NO:__; Invitrogen, Carlsbad, CA). The affinity sequence may be a hexa-histidine tag as supplied, for example, by a pBAD/His (Invitrogen) or a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host. Alternatively, the affinity sequence may be a hemaglutinin (HA) tag when mammalian host cells, for example COS-7 cells, are used. The HA tag corresponds to an antibody defined epitope derived from the influenza hemaglutinin protein (Wilson et al., 1984, *Cell 37*:767).

In the claims:

Please amend claims 96, 104, 107 and 108 to read as follows:

96. (Amended) The method of claim 92 wherein the first isolated recombinant polypeptide comprises a cyclophilin polypeptide fused to an additional polypeptide that is selected from the group consisting of polyhistidine, polylysine, a

haemagglutinin epitope tag, a DLYDDDDK [SEQ ID NO:__] epitope tag, a DYKDDDDK [SEQ ID NO:__] epitope tag, a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

104. (Amended) The method of claim 92 wherein the second isolated recombinant polypeptide comprises a human adenine nucleotide translocator polypeptide or variant thereof that is fused to an additional polypeptide selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, a DLYDDDDK [SEQ ID NO:__] epitope tag, a DYKDDDDK [SEQ ID NO:__] epitope tag, a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

107. (Amended) The method of claim 106 wherein the second isolated recombinant polypeptide comprises a human adenine nucleotide translocator polypeptide or variant thereof that is fused to a polypeptide selected from the group consisting of a DLYDDDDK [SEQ ID NO:__] epitope tag and a DYKDDDDK [SEQ ID NO:__] epitope tag, and wherein the antibody specifically binds to at least one polypeptide selected from the group consisting of the human adenine nucleotide translocator polypeptide, the XPRESS™ epitope tag and the FLAG® epitope tag.

108. (Amended) The method of claim 92 wherein the first isolated recombinant polypeptide comprises human cyclophilin D and wherein the sample which comprises the second isolated recombinant polypeptide comprises at least one submitochondrial particle isolated from a *T. ni* cell that expresses a recombinant human adenine nucleotide translocator-3 polypeptide fused to a DLYDDDDK [SEQ ID NO:__] epitope tag.

REMARKS

Reconsideration of the present application in view of the above amendments and the following remarks is respectfully requested. Claims 96, 104, 107 and 108 have been amended, without limitation, to more distinctly point out and particularly claim subject matter which applicants regard as the invention. Support for the amendment may be found in the specification, for example, at page 28, line 24 through page 29, line 5; at page 70, lines 3-10; at page 82, lines 2-4; at page 83, lines 22-27; at page 87, lines 1-2; at page 102, lines 6-11; at page 117, lines 20-23. No new matter has been added.

Claims 1-91 have previously been canceled and claims 92-139 are currently pending in the application. In response to a Restriction Requirement mailed October 12, 2001, applicants elected Group IV, claims 37-61 and 63-67, in a Response filed on November 13, 2001. In the Office Action mailed on January 30, 2002, to which the present amendment and response are directed, it is asserted that the elected method claims correspond to new claims 92-108 and that claims 92-108 are being examined. Applicants respectfully request clarification with regard to the status of pending claims 109-139, which have not been expressly canceled, and therefore applicants respectfully request that claims 109-139 be withdrawn by the Examiner without prejudice to the filing of any related continuation, continuation-in-part or divisional application.

Applicants respectfully request reconsideration of instant claims 92-108, which are currently pending and under examination in the present application, and which are directed to *a method of identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide*. In this regard, and as discussed in greater detail below, applicants are confused by the Action in its assertion of rejections of the instant claims under 35 U.S.C. §103, which rejections make reference, *inter alia*, to a method for screening an agent that can alter mitochondrial permeability transition (MPT), to a method comprising contacting a host cell with a candidate agent, to a method comprising comparing a level of energy transfer, and to altering cell survival. In particular, applicants respectfully point out that the recitations "mitochondrial

permeability transition", "MPT", "host cell", "energy", "energy transfer", "survival" and "cell survival" are all absent from the claims currently under examination. Hence, applicants respectfully submit that the rejections in the Action under 35 U.S.C. §103 appear to be inapposite to the claims currently under examination. For the Examiner's convenience, the attached "Version with Markings to Show Changes Made" includes all claims currently under examination.

REJECTIONS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

In the Office Action, claims 96, 104, 107 and 108 were rejected under 35 U.S.C. §112, second paragraph as indefinite. In particular, it is alleged that the instant claims use the trademarks "XPRESS" and "FLAG" to identify or describe polypeptide tags of uncertain scope, rendering these recitations indefinite.

Applicants respectfully traverse these grounds of rejection and submit that they are moot in view of the present amendment. The trademarks recited in the instant claims (FLAG® and XPRESS™) have been deleted and replaced with actual epitope tag amino acid sequences DYKDDDDK (SEQ ID NO:__) and DLYDDDDK (SEQ ID NO:__), respectively (*see, e.g.*, specification at page 28, line 24 through page 29, line 5; at page 70, lines 3-10; at page 82, lines 2-4; at page 83, lines 22-27; at page 87, lines 1-2; page 102, lines 6-11; at page 117, lines 20-23). Applicants respectfully submit that amendment of the instant specification and claims to include amino acid sequences for the FLAG® and XPRESS™ epitope tags is proper and does not add new matter, because the peptide epitope sequences were disclosed in the references cited by the specification (for FLAG® [SEQ ID NO:__], see, *e.g.*, U.S. 5,011,912 and Hopp et al., 1988 *BioTechnol.* 6:1204, cited, *e.g.*, at specification page 28, lines 27-29; for XPRESS™ [SEQ ID NO:__], see, *e.g.*, "pBAD/His", the unique identifier for a vector bearing the insert encoding SEQ ID NO:__, for which vector map and sequence are available from Invitrogen, Carlsbad, CA, (a manufacturer well known to the art) and which are described in catalogs of this supplier, cited, *e.g.*, at specification page 87, line 2), which publications are incorporated by reference in the instant specification (*e.g.*, at page 15,

lines 14-17). Moreover, FLAG® and XPRESS™ epitope tags were available on the market and were known generally to those having ordinary skill in the art of molecular biology, and had a fixed and definite meaning at the time the subject application was filed (see, *e.g.*, *In re Gebauer-Fuelnegg*, 50 U.S.P.Q. 125, 128, C.C.P.A. 1941).

Accordingly, applicants respectfully submit that the claims satisfy the requirements of 35 U.S.C. §112, second paragraph, and therefore request that these rejections be withdrawn.

REJECTIONS UNDER 35 U.S.C. SECTION 103

A. Claims 92-94, 97-103 and 105 are rejected under 35 U.S.C. Section 103 as allegedly obvious over Marban et al. (U.S. Patent No. 6,183,948) in view of Luban et al. (U.S. Patent No. 5,773,225) and further in view of Anderson et al. (U.S. Patent No. 6,140,067). In particular, the Action (page 4) alleges that Marban et al. teach a screening assay for an agent that alters mitochondrial permeability transition comprising steps that include contacting a host cell with a candidate agent and an inducer of MPT, exposing the cell to excitation energy, detecting energy transfer (ET) between first and second ET molecules and comparing the energy transfer level to a reference level generated in the absence of candidate agent.

The Action concedes that Marban et al. fail to provide a host cell which comprises first and second nucleic acid expression constructs encoding, respectively, a mitochondrial permeability transition pore component fused to a first ET molecule and a cyclophilin polypeptide fused to a second ET molecule. The Action asserts, however, that Luban et al. teach a method wherein the host cell comprises a first nucleic acid expression construct comprising a promoter operably linked to a polynucleotide encoding a first ET molecule and a second nucleic acid expression construct comprising a promoter operably linked to a polynucleotide encoding a cyclophilin polypeptide fused to a second ET molecule (Action, page 5, lines 5-11). The Examiner alleges further that in the method of Luban et al., "binding of the polypeptide to the cyclophilin polypeptide results in detectable energy transfer between the first and second energy transfer molecules

(Figures 1-2 and Column 6, line 40 to column 9, line 25)" (Action at page 5, lines 11-12). The Action then alleges that Luban et al. teach that the method disclosed by Luban et al. can be "extended to assays using protein expressed in baculovirus, tissue culture cells or Gag purified from virus" (Action, page 5, lines 16-17), from which a person having ordinary skill in the art would have found it obvious to combine the cyclophilin fusion construct of Luban et al. with the screening method for an agent that can alter MPT of Marban et al. to arrive at the claimed invention.

The Action also asserts (pages 6-7) that Anderson et al. teach the following: detection of agents that alter MPT, ANT-cyclophilin binding in mitochondrial membranes, comparing altered mitochondrial function in the presence and absence of a candidate agent, a method for altering cell survival, and a nucleotide construct encoding a MPT pore component polypeptide fused to a polynucleotide wherein binding of the MPT pore component polypeptide to the cyclophilin polypeptide results in detectable ET between first and second ET molecules. The Action then alleges that a person having ordinary skill in the art would have found it obvious to substitute the MPT pore component polypeptide of Anderson et al. in the screening method of Marban et al. modified in view of Luban et al.

Applicants respectfully traverse these grounds for rejection and submit that the prior art, including the references cited by the Action alone or in combination, fails to teach or even remotely suggest the claimed invention. As noted above, applicants respectfully submit that the thrust of the Action appears to be directed to subject matter quite different from that of the currently pending claims in the instant application, such that applicants respectfully request reconsideration of the instant claims.

As disclosed in the specification and recited in the claims, the present invention is directed in pertinent part to a method of identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide, comprising: (a) contacting, in the absence and presence of a candidate agent, (i) a first isolated recombinant polypeptide comprising a cyclophilin polypeptide or variant thereof with (ii) a sample comprising a second isolated recombinant polypeptide that comprises a

recombinant human adenine nucleotide translocator polypeptide or variant thereof, under conditions and for a time sufficient to permit the cyclophilin polypeptide, the adenine nucleotide translocator polypeptide and the candidate agent to interact; and (b) comparing a level of binding of the first isolated recombinant polypeptide to the second isolated recombinant polypeptide in the absence of the candidate agent to the level of binding of the first isolated recombinant polypeptide to the second isolated recombinant polypeptide in the presence of the candidate agent, wherein a decreased level of binding in the presence of the agent indicates an agent that inhibits binding of an adenine nucleotide translocator polypeptide to a cyclop[illegible]polypeptide and wherein an increased level of binding in the presence of the age[illegible]ndicates an agent that enhances binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide, and therefrom identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide.

Applicants submit that the prior art, including the references cited by the Examiner, fails in any way to suggest a method comprising the *combination* of using (i) an isolated *recombinant* cyclophilin polypeptide, and (ii) a sample comprising an isolated *recombinant* adenine nucleotide translocator polypeptide, in a binding assay to screen for agents that increase or decrease binding between the two recombinant polypeptides. In particular, the prior art fails to contemplate, nor has the prior art been shown by the Examiner to have been in possession of, an isolated recombinant ANT polypeptide, for use in a combination according to the claimed method. Applicants therefore respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness.

Marban et al. merely disclose excitation and detection of endogenous cellular flavoprotein fluorescence as a mitochondrial redox marker, or of the fluorescence emission of a cellularly introduced fluorescent marker such as TMRE (Col. 14, lines 16-28, 39-42, 45-57). However, Marban et al. absolutely fail to teach or suggest a binding assay in which an isolated recombinant cyclophilin polypeptide is contacted with an isolated recombinant ANT polypeptide. Additionally, applicants submit that this deficiency of Marban et al. simply cannot be remedied by Luban et al. and/or by Anderson et al., neither of which contemplate contacting the combination of an isolated

recombinant cyclophilin polypeptide and an isolated recombinant ANT polypeptide. As an aside, applicants submit that contrary to the assertions in the Action, the fluorescent signals described by Marban et al. are not the products of energy transfer between first and second ET molecules, nor are these fluorescent signals the products of ET between ET fusion proteins such that ET results from binding of a MPT pore component polypeptide to a cyclophilin polypeptide.

Luban et al. merely disclose a specific application of the well known yeast two-hybrid screening system for identifying interacting gene products, detected by colorimetric (and not energy transfer, as alleged in the Action) determination of the reporter gene product beta-galactosidase-mediated conversion of the β-galactosidase (β-Gal) substrate X-Gal, to identify HIV gag protein binding to cyclophilin A or cyclophilin B. Similarly, Figs. 1-2 of Luban et al. (Figs. 1-2 cited in the Action) merely depict GAL4-cDNA fusion construct joint sequences, and an activity map of the apparent cyclophilin-binding properties of several mutant gag-GAL4 fusion constructs, again using colorimetric and not ET detection. Applicants submit, however, that nowhere do Luban et al. teach or in any way suggest a binding assay according to the present invention wherein an isolated recombinant cyclophilin polypeptide is contacted with an isolated recombinant ANT polypeptide. Moreover, the Action fails specifically to point to any source in the prior art that can remedy this deficiency of the combination of Marban et al., Luban et al. and Anderson et al., particularly with regard to the failure of the art to contemplate an isolated recombinant ANT polypeptide for use in the subject invention method.

Additionally, applicants submit that Luban et al. teach away from the presently claimed invention because the constructs of Luban et al. encode polypeptides that are necessarily targeted to localize to the cell nucleus in order to complement one another to reconstitute a functional β-Gal transcription complex. By way of contrast, the isolated recombinant ANT and cyclophilin proteins according to the instant claims would not be usefully targeted to a cell nucleus, which is a requirement for the constructs of Luban et al. Instead, ANT is a *mitochondrial* polypeptide (as also is cyclophilin D), that would not reasonably be expected to possess a nuclear localization sequence. Hence,

applicants traverse the allegation in the Action (p. 5) that the statement of Luban et al. suggesting broad applicability of the assay of Luban et al. (Luban et al., col. 5, lines 22-24) would have motivated the ordinarily skilled artisan to use the construct of Luban et al. (*i.e.,* obligatorily nuclear targeted) in the method of Marban et al. Marban et al. teach a cell-based assay that does not rely upon nuclearly targeted proteins, hence applicants submit a person having ordinary skill in the art would lack any reasonable expectation of success from combining Marban et al. with Luban et al. to arrive at the subject invention method of the instant claims, which method does not require a cell-based assay nor recite any intracellular or organellar compartment.

Moreover, both Marban et al. and Luban et al. fail in any way to suggest any relevance of any cyclophilin polypeptide to the disclosure of Marban et al., and the Action has failed to establish such motivation, for reasons discussed herein. Accordingly, applicants submit that absent the disclosure of the present application, there would have been no motivation to combine the teachings of the references cited by the Examiner, nor has the Action specifically pointed to any such source of motivation, in view of the remarks submitted herewith.

Furthermore, applicants submit that contrary to the assertions found in the Action, a person having ordinary skill in the art would not have been motivated, with any reasonable expectation of success, to substitute the cyclophilin fusion constructs of Luban et al. in the method of Marban et al. to arrive at the present invention. First, the Action refers to the alleged "cyclophilin D containing fusion nucleic acid construct of Luban et al." (Action at page 5, last three lines), but applicants note that cyclophilin D is nowhere mentioned by Luban et al., nor do Luban et al. offer any suggestion regarding applicability of the disclosure found therein to any mitochondrial polypeptides such as ANT or cyclophilin D. Additionally, Marban et al. are silent with regard to cyclophilin D or any cyclophilin, and thus the collective teachings of the cited references simply cannot be portrayed as suggesting any desirability of combining the cited disclosures to arrive at a method featuring the recited combination of isolated recombinant cyclophilin and ANT polypeptides. Second, even assuming *arguendo* that the disclosure in Luban et al. pertaining to cyclophilins A and B were to be applied generally to include

(mitochondrial) cyclophilin D, as the Examiner appears to be suggesting, applicants submit the prior art teaches away from any such motivation to combine Luban et al. with Marban et al. or with any other reference. Specifically, cyclophilins A and B are non-mitochondrial isoforms, in contrast to the sole mitochondrial isoform cyclophilin D known to the art (see, *e.g.,* specification at page 7, lines 23-26). Furthermore, the Action asserts that an ordinary practitioner would have been motivated to use the cyclophilin construct of Luban in the MPT screen of Marban et al., but applicants submit that Luban et al. are utterly silent with regard to any relevance whatsoever that any cyclophilin constructs described by Luban et al. may have to mitochondria or to any mitochondrial function (*e.g.,* MPT). Thus, the disclosure of Luban et al. contains absolutely no teaching or suggestion pertaining to mitochondria at all, and certainly not to the subject invention method for identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide.

With regard to Anderson et al., applicants submit that citation of this reference in the Action is merely cumulative with disclosure found, for example, at pages 6-8 of the instant application. The passages in Anderson that are pointed to by the Action (*e.g.,* Action at pages 6-7) merely disclose that ANT and cyclophilin D are mitochondrial membrane components that are capable of binding interactions and that may, under certain conditions, provide indicators of altered mitochondrial function as described by Anderson et al. This disclosure in no way, however, suggests the first and second isolated recombinant polypeptides that are described in the present application and recited in the instant claims, and applicants submit that the Examiner has pointed to no suggestion by Anderson et al. that ANT-cyclophilin binding might be detected using an isolated recombinant cyclophilin polypeptide in combination with an isolated recombinant ANT polypeptide according to the instant claims.

Applicants are further puzzled by, and traverse, the assertions in the Action at page 8, lines 2-4, that the highly generalized statement which forms the first sentence of the abstract in Anderson et al. would provide the express motivation to a person having ordinary skill in the art to substitute the MPT polypeptide of Anderson et al. in the screening method of Marban et al. in view of Luban et al. As discussed herein,

Marban et al. merely monitor cellular fluorescence redox indicators while Luban et al. describe a yeast dihybrid screen that has nothing to do with ANT or with recombinant ANT or with any cyclophilin. Thus, in the absence of guidance from any of the references with regard to which of the myriad "MPT polypeptides" or mitochondrial "indicators" of Anderson et al. might be selected, applicants submit that only through the use of impermissible hindsight in view of the present application could an ordinarily skilled artisan divine what specifically are the "improved screening assays . . . by comparing the levels of one or more indicators of altered mitochondrial function" to arrive at the claimed invention. For instance, and as noted herein, neither Anderson et al. nor any of the other cited references in any way contemplate the use of an isolated, recombinant ANT polypeptide, nor has the Examiner provided any evidence of same in the prior art.

The specific disclosures in Marban et al., Luban et al. and Anderson et al., which are called out by the Action as noted above, are in fact essentially irrelevant to the subject matter of the claims currently under examination. Hence, applicants respectfully submit that the Examiner has failed to establish a case of *prima facie* obviousness, where the references cited by the Action, alone or in combination, fail to teach or suggest the subject matter of the instant claims. (*See In re Mayne*, 104 F.3d 133, 1341-43, 41 U.S.P.Q.2d 1451 (Fed. Cir. 1997) (PTO has the burden of showing a *prima facie* case of obviousness.)). The Examiner must show (1) that the combined references teach or suggest all claim limitations; (2) that the references provide some teaching, suggestion, or motivation to combine or modify the teachings of the prior art to produce the claimed invention; and (3) that the combined teachings of the references indicate that by combining the references, a person having ordinary skill in the art will achieve the claimed invention with a reasonable expectation of success. When rejection of claims depends upon a combination of prior art references, a teaching, motivation, or suggestion to combine the references must exist. (*See In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453 (Fed. Cir. 1998)).

Applicants are therefore somewhat puzzled by the emphasis in the Action, as described above, on the teachings of Marban et al. as they pertain to inducing MPT in

a host cell and detecting "energy transfer", which applicants submit are not teachings found anywhere in Marban et al., Luban et al. or Anderson et al. and which, in any event, have no relevance to the instant claims. For similar reasons, applicants find unclear the purposes of the assertions in the Action with regard to teachings in Luban et al. that relate to a host cell comprising first and second nucleic acid expression constructs which each encode an "energy transfer" molecule, which teachings applicants submit are not teachings found in Luban et al. and which, again, have no relevance to the instant claims.

In view of the foregoing, applicants respectfully submit that the instant claims satisfy the requirements of 35 U.S.C. §103 and request that these rejections be withdrawn.

B. The Action also rejected claims 92-94 and 96-105 under 35 U.S.C. §103 over Marban et al. in view of Luban et al. further in view of Anderson et al. and further in view of Briggs et al. (U.S. 6,211,440). Specifically, Briggs et al. are alleged to teach ET molecules that are green fluorescent proteins (GFP).

Applicants respectfully traverse these grounds for rejection. The present invention is directed in pertinent part to a method of identifying an agent that alters binding of an ANT polypeptide to a cyclophilin polypeptide, as discussed above, including such method wherein the isolated recombinant cyclophilin polypeptide is fused to an additional polypeptide that is selected from the recited set of detectable polypeptide domains, including GFP. The deficiencies of Marban et al., Luban et al. and Anderson et al. are also discussed above, and applicants submit that as described herein, the deficiencies of any or all of these references are not remedied by Briggs et al.

Briggs et al. simply disclose GFP as one of numerous useful labels for detecting nucleic acids that are disclosed by Briggs et al., but the teachings of Briggs et al. are irrelevant to the subject invention screening assay for an agent that alters binding of an ANT polypeptide to a cyclophilin polypeptide. Applicants submit that this reference is inapposite to the present invention, *inter alia*, because Briggs et al. teach using GFP merely as a post-biosynthetically, covalently attached fluorescent protein label for a nucleic acid, and not as an additional polypeptide that is fused in-frame to the

recited isolated recombinant cyclophilin polypeptide, for example, to yield a cyclophilin-GFP fusion protein. Briggs et al., alone or in combination with any other references cited by the Examiner, in no way contemplate the presently claimed invention. On the contrary, the passage from Briggs et al. as cited at page 9 of the Action is simply a generic statement concerning useful detectable labeling moieties, but the Action fails to point to any suggestion whatsoever in Briggs et al. that GFP as described therein might usefully be fused to a cyclophilin domain of an isolated recombinant polypeptide for use in the subject invention method.

Accordingly, no *prima facie* case of obviousness has been established where, for reasons elaborated herein, there has been no showing that the prior art appreciated or in any way contemplated the use of two isolated recombinant polypeptides, ANT and cyclophilin, in a method to identify an agent that alters binding according to the presently claimed invention. Accordingly applicants request that these rejections under 35 U.S.C. §103 be withdrawn.

C. Claims 92-95, 97-103 and 105-106 stand rejected under 35 U.S.C. §103 over Marban et al. in view of Luban et al. further in view of Anderson et al. further in view of Halestrap et al. (1998 *Biochim. Biophys. Acta.* 1366:79). The assertions in the Action with respect to Marban et al. in view of Luban et al. further in view of Anderson et al. are described above. The Action asserts further that Halestrap et al. teach the claimed method wherein cyclophilin A polypeptide is used (claim 95), and wherein the detection reagent is an antibody (claim 106).

Applicants respectfully traverse these grounds for rejection. As also described above, the present invention is directed in pertinent part to a method of identifying an agent that alters binding of an ANT polypeptide to a cyclophilin polypeptide, comprising in pertinent part contacting an isolated recombinant cyclophilin polypeptide and an isolated recombinant adenine nucleotide translocator (ANT) polypeptide, in the absence and presence of a candidate agent.

The deficiencies of the combination of Marban et al., Luban et al. and Anderson et al. are discussed above. Contrary to the assertion in the Action, Halestrap et

al. fail to provide any disclosure relating to a binding assay in which an isolated recombinant ANT polypeptide is permitted to bind to an isolated recombinant cyclophilin A polypeptide, regardless of whether an antibody is used as a detection reagent. On this point, the Action makes specific reference to page 80, section 2.1 of Halestrap et al., but applicants submit that Halestrap et al. therein merely describe assays of cyclophilin A peptidyl-prolyl cis-trans isomerase activity, which neither teaches nor suggests assays of binding between isolated recombinant ANT and recombinant cyclophilin polypeptides. Halestrap et al. also describe effects of cyclophilin A on MPT, which applicants respectfully submit are not and need not be assays of isolated recombinant ANT binding to isolated recombinant cyclophilin binding according to the present invention. Nowhere, therefore, do Halestrap et al. teach or suggest a direct and straightforward binding assay between isolated recombinant ANT and isolated recombinant cyclophilin, as is provided by the present invention. In fact, Halestrap et al. disclose only the use of non-recombinant ANT from detergent solubilized mitochondrial membranes, which ANT has different properties from isolated recombinant ANT polypeptides of the present invention; for instance, the non-recombinant ANT of Halestrap et al. cannot be engineered into a recombinant fusion protein having any of the attributes of ANT fusion proteins described in the present specification and recited in the instant claims. For reasons also given above, neither Halestrap et al. nor any of the other references cited in the Action contemplate the subject invention method, and in particular, a method in which an isolated recombinant ANT polypeptide is used. Accordingly, where the disclosure of Halestrap et al. is limited to indirect measurement of ANT-Cyp interactions, as evidenced by the assertions in the Action concerning disclosure by Halestrap et al. of MPT assays but not by any teaching or suggestion directed to a direct binding assay using isolated recombinant ANT, applicants submit that the present invention is not obvious over the prior art.

Applicants therefore respectfully submit that the Action has failed to establish a *prima facie* case of obviousness, and request that the rejection be withdrawn.

All of the claims remaining in the application are now clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

Anne N. Murphy *et al.*

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